SECTION SIX

The history of use or other evidence of safety establishing that the dietary ingredient creatine from creatine ethyl ester HCl when used under the conditions recommended or suggested in the labeling of dietary supplement products will reasonably be expected to be safe and which is the basis on which the distributor of creatine from creatine ethyl ester HCl has determined that the use of creatine from creatine ethyl ester HCl is reasonably expected to be safe. See 21 CFR Section 190.6(b)(4).

INTRODUCTION

Creatine ethyl ester HCl as the source of creatine is a structurally related chemical analog of creatine. The difference between creatine and creatine ethyl ester HCl is that the carboxylic acid group of creatine has been masked through the formation of an ester linkage. The masking of the carboxylic acid, results in a creatine-based compound with both increased aqueous solubility and enhanced membrane partitioning compared to standard creatine monohydrate. (See Vennerstom JL, Miller DW. Creatine Ester Pronutrient Compounds and Formulations. International publication number WO 02/221535A1. World Intellectual Property Organization, 21 March 2002, as Attachment 6.)

Creatine has been available to retail consumers as a dietary supplement from various sources since 1992 and would be a "grandfathered" dietary ingredient under Section 413(c) of the FDC Act. From initial marketing until now, no significant health concerns have been identified in either controlled human studies, or acute and sub-acute toxicity studies in laboratory animals. Creatine ethyl ester HCl is a combination of creatine and ethanol which has been shown in ChemPharma laboratory study (see Attachment 24) to enter the body following oral administration as the individual constituents creatine and ethanol (i.e. ethyl alcohol) Ethyl Alcohol is listed in the "Food Chemicals Codex" 4th ed. (1996 p136) and is affirmed as GRAS by FDA at 21 CFR Section 184.1293 as an ingredient used "as an antimicrobial agent . . . not to exceed 2.0 percent by product weight." It is also subject to a food additive regulation at 21 CFR Section 169.175 as component of vanilla extract.

Additionally, Triethyl citrate found at 21 CFR Section 184.1911 is the triethyl ester of citric acid. It is prepared by esterifying citric acid with ethyl

alcohol. FDA also recognizes the dietary supplement status of botanical extracts in ethanol. See 21 CFR Section 101.36(b)(3)(ii)(B).

Attachments 7 and 8 indicate creatine was produced for sale by Pfanstiehl laboratories as early as 1972 and was subject to trademark applications by marketing companies in 1993 that allege existing commercial use in commerce of creatine. Based on that prior commercial use, creatine could be considered a "grandfathered dietary ingredient" under Section 413(c) of the FDC Act. Additionally, ethyl alcohol is GRAS and a component of approved food additives. For that reason, we would conclude that the amino acid creatine from the source product of both creatine and ethyl alcohol (i.e. creatine ethyl ester) would clearly meet the definition of a dietary supplement found at Section 201 (ff)(D)(E) of the FDC Act.

Safety of Creatine from Creatine Ethyl Ester HCl

Numerous studies evaluating the relative safety of creatine supplementation for healthy adults have been published. (See Attachments 9-15.) These human studies include both short and long term studies, and have established that dietary supplementation with creatine is not associated with any adverse health effects. No differences were noted in serum markers of liver or kidney function between groups using creatine supplementation as compared to placebo. (See Attachments 16-19.) Two case reports of kidney dysfunction following creatine use exist within the medical literature. However, neither case report was able to demonstrate a causative relationship with creatine supplement use. (See Attachments 20 and 21.) Creatine is an accepted ergogenic supplement in all major athletic organizations, including IOC, NCAA and other major sports organizations. (See Attachments 22 and 23.)

Ethanol is a well-established component of the human diet. The supplementation of a daily dosage of creatine from creatine ethyl ester of 5 grams contains 1.47 grams of ethanol. A single cocktail made with 40 proof liquor contains as a matter of comparison to regular human use contains 16.8 grams of ethanol. This normal human use is more that 10 times the amount of ethanol provided in the recommended daily dosage of creatine from creatine ethyl ester HCl.

Results reported in the September 2002 75 day premarket notification by PNT indicated that in a long term human trial involving five subjects, that there was one subject with a slightly elevated serum creatinine level (1.7 mg/dl), whereas normal levels are typically 0.8-1.5

mg/dl. This matter was cited by FDA as one concern regarding the position that creatine ethyl ester is reasonably expected to be safe. The elevated creatinine level mentioned above is only 13% above the normal range, and given the limited number of subjects and the nature of the analytical methodology, it is arguable this finding would not represent a clinically significant increase or cause for concern. Additionally, it has been established in this 75 day premarket notification that the safety of creatine and ethanol is the relevant issue because both constituents will result in the body under normal recommended use of creatine from creatine ethyl ester HCl.

ChemPharma Int'l. final report of the study entitled "Identification and Quantitation of Bioavailable [^{14}C]Compounds Present it the Blood and Urine of Rats Following Oral Administration of a Single Dose of [^{14}C]Creatine Ethyl Ester" is included as Attachment 24.

This ChemPharma study was designed with the source cooperation and assistance of personnel at the Center for Food Safety and Applied Nutrition (CFSAN) to examine the form in which the ingredient creatine ethyl ester HCl enters the mammalian body (rat) (after proper dosing with this ingredient) in which the creatine and ethanol moiety were both radiolabeled. A review of the attached study establishes that following oral administration to mammals, the source ingredient creatine ethyl ester HCl is immediately dissociated to creatine and ethanol. The ethanol is rapidly metabolized and eliminated as CO_2. The creatine mostly is rapidly distributed to tissues (e.g. skeletal muscle), then excreted in the urine as creatine's well established metabolite, creatinine.

At the guidance of Dr. Linda Pellicore of CFSAN, a second study was undertaken by ChemPharma Int'l. (Pharmacokinetics and Identification of [^{14}C]Compounds Present in the Plasma of Rats Following the Oral Administration of a Single Dose of [^{14}C]Creatine from [^{14}C]Creatine Ethyl Ester Hydrochloride, see Attachment 25). The protocol and design of this study was confirmed in a letter addressed to Dr. Pellicore that was received by FDA on October 21, 2004. Although the agency initially requested that we demonstrate elimination of radiocarbon and establish material balance, it should be noted that neither are a component of this second study. They were established in the earlier study addressed above. The pharmacokinetics of the conversion of the CEE to creatine and its metabolites is specifically the topic of this study. For that reason, this second study involved the pharmacokinetics of creatine in the plasma of rats following oral administration of [^{14}C]Creatine associated with ethanol (i.e. the ester vehicle of administration). The radiolabelled ester was synthesized at a

very high specific activity (31,468,827 dpm/mg; 2.13 mCi/mmol) to achieve blood levels of radiocarbon that were sufficiently high to ensure accurate identification and quantitation (using radiometric HPLC analysis) of the administered form of the compound along with its dissociated species creatine and its primary metabolite creatinine following absorption from the gastro-intestinal tract. Since the earlier study discussed above had indicated that the ester form of creatine dissociates extremely rapidly into creatine and ethanol following oral administration and absorption (ChemPharma Int'l., LLC Study No. 145, Identification and Quantitation of Bioavailable [^{14}C]Compounds Present it the Blood and Urine of Rats Following Oral Administration of a Single Dose of [^{14}C]Creatine Ethyl Ester, see Attachment 24), this study, using very high specific activity [^{14}C]Creatine associated with ethanol, was conducted to definitively establish the identity and time course of the products of dissociation and metabolism.

Results from this study included as Attachment 25 establish that, following oral administration the [4-^{14}C] Creatine Ethyl Ester is rapidly absorbed from the gastro-intestinal tract, with radioactivity being detected in plasma within three minutes following administration. The peak concentration of the associated form (ester) occurred at five minutes following administration, and accounted for less than 15% of the total radioactivity present in the plasma. This indicates that its dissociation into creatine and ethanol is rapid and nearly complete within this initial five-minute period. At ten minutes following administration, approximately 2% of the total radioactivity present in plasma was in the associated ester form, with none detected after the ten-minute time point. The rapid dissociation of the ethyl ester in the plasma was accompanied by a concurrent rapid increase in creatine and creatinine levels as suggested in the earlier study (see Attachment 24). The peak level of creatine in plasma was equivalent to about 12% of the total radiocarbon present, and occurred at approximately thirty minutes following administration. The concentration gradually declined, and gave rise to creatinine levels that increased to approximately 80-90%, where they remained throughout the duration of this study period. This pharmacokinetic profile is expected, since creatinine is the known major metabolite of creatine.

In summary, when creatine is orally administered in its ethyl ester form, it rapidly dissociates upon absorption from the gastro-intestinal tract into the bloodstream, giving immediate rise to creatine *per se* which is subsequently metabolized primarily to creatinine which is excreted in the urine as demonstrated in the previous study (see Attachment 24). Therefore, the bioavailable form of creatine, when administered orally as the ester form, is creatine rather than the intact (associated) ethyl ester,

and confirms the conclusions drawn in the earlier report (see Attachment 24).

The professional credentials of the author and others involved with these studies are included as Attachment 26.